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Research Article

Association of Markers of Non-Alcoholic Fatty Liver Disease with Cardiac Structure and Function in a General Population of Adolescents

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- Adiposity

Abstract

Background and Aims: Little evidence exists from general population studies examining the association of non-alcoholic fatty liver disease (NAFLD) with cardiac structure and function, independent of adiposity.

Methods: Cross-sectional study of a general population of adolescents (mean age 17 years). We estimated associations of 1. Blood-based markers of NAFLD: alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) (N = 1,440) 2. Ultrasound (USS) diagnosed NAFLD and shear velocity (N = 654) with echocardiography-based measures of cardiac structure and function.

Results: ALT, AST, GGT and shear velocity were positively associated with left ventricular mass index [LVMI (g/m^{2.7})], mean difference (95% confidence intervals(CI)) per 10U/L for blood-based markers and per m/sec for shear velocity: 0.56 (0.28, 0.83), 0.40 (0.02, 0.77), 0.59 (0.36, 0.81) and 0.61 (0.13, 1.09) when adjusting for age, sex, social class, pubertal status, smoking, and alcohol intake and with left atrial size index: 1.39% (0.66%, 2.13%), 1.28 (0.30, 2.28), 1.01 (0.43, 1.59) and 2.56 (1.21, 3.93) respectively. After additional adjustment for fat mass, only GGT remained associated with LVMI: 0.29 (0.09, 0.50). GGT and shear velocity were inversely associated with peak myocardial wall velocity in systole in confounder-adjusted models including fat mass. ALT, AST and GGT were positively associated with left ventricular diastolic diameter in age and sex-adjusted model, but associations attenuated in confounder-adjusted models. No associations between measures of NAFLD and relative wall thickness, mid wall fractional shortening, ejection fraction, E/e', mitral E/A, and e' were found and there was no difference in any measures of cardiac structure and function between adolescents with (N = 13) and without USS fatty liver (N = 641).

Conclusions: We found no robust evidence that markers of NAFLD are associated with cardiac structure or function independent of adiposity in a general adolescent population.

ABBREVIATIONS

LVMI: Left Ventricular Mass Indexed to height^{2.7}; RWT: Left Ventricular Relative Wall Thickness; LAI: Left Atrial Size Indexed to height^{2.7}; E/e: Diastolic Transmitral flow velocity; E/A: Ratio of Early Late Transmitral Flow Velocity; e: Early Diastolic Velocity

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of mortality amongst adults with non-alcoholic fatty liver disease (NAFLD) [1,2]. NAFLD is associated with greater carotid intima media thickness [3], higher blood pressure [4-6], adverse cardiac structure and function [7-10], dyslipidemia [6] and

hyperglycaemia [4], even after adjusting for established CVD risk factors including whole body adiposity and lipid levels. Consequently, it has been suggested that NAFLD is implicated in the development of CVD through systematic processes such as insulin resistance, low-grade inflammation, and myocardial lipid accumulation due to increased free fatty acid flux [11,12]. However, other reports suggest that the association between NAFLD and cardiac structure and function is not independent but driven by a common cause - adiposity [13,14].

Measures of cardiac structure, diastolic and systolic function in childhood track through to adulthood [15,16] and are predictors of CVD later in life [17]. Adiposity is closely associated with both

NAFLD [18] and altered cardiac function [19-21] and therefore it is plausible that associations between the latter are confounded by adiposity. The majority of studies that have reported adverse cardiac structure and function in adolescents with NAFLD have been conducted in selected obese populations [22,23] and there is a paucity of evidence from the general adolescent population.

We examined associations of markers of NAFLD: Alanine Amino Transferase (ALT), aspartate amino transferase (AST) and gamma-glutamyltransferase (GGT) and ultrasound scan (USS) assessed fatty liver and shear velocity (a marker of liver fibrosis/stiffness), with measures of cardiac structure and function in a general population sample of adolescents. We also aimed to examine whether any associations found were confounded by adiposity.

METHODS

Study participants

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a prospective, population-based birth cohort study that recruited 14,541 pregnancies, of which there were 13,867 live births from 13,761 women in Avon, UK, with expected dates of delivery 1st April 1991 to 31st December 1992 (<http://www.alspac.bris.ac.uk>) [24,25]. Please note that the study website contains details of all the data that is available through a fully searchable data dictionary.

The cohort has been followed-up since birth with questionnaires, and from the age of seven participants has been seen regularly in clinic; the most recent of these was the 17-18 year clinic assessment. This clinic assessment was attended by 5,206 participants, and included two separate sub-studies. In one, liver ultrasounds (USS) were conducted and in the other, echocardiography measures were taken. To be eligible for the present study, participants had to have blood-based markers of NAFLD and/or participated in the echocardiography sub-study. Singletons and one randomly chosen twin from twin pairs was included (see Figure 1).

In order to remove any effect due to excess alcohol intake, which is associated with fatty infiltration in the liver, consistent harmful alcohol drinkers were removed from the analysis. Information on participant's alcohol consumption was obtained by questionnaires administered at age 16 years, and again at 17 years (at the same time as the USS assessment), using the Alcohol Use Disorders Identification Tests (AUDIT) questionnaire [26]. Participants answered 10 questions about their alcohol consumption, and from their responses, a score between 0 and 20 was derived. A score over 16 is classified as harmful alcohol consumption [26]. Consistent harmful alcohol drinkers were defined by a score of 16 or greater at both 16 years and 17 years. No participants had a known history of jaundice or hepatitis, or were taking medications or receiving treatment that would

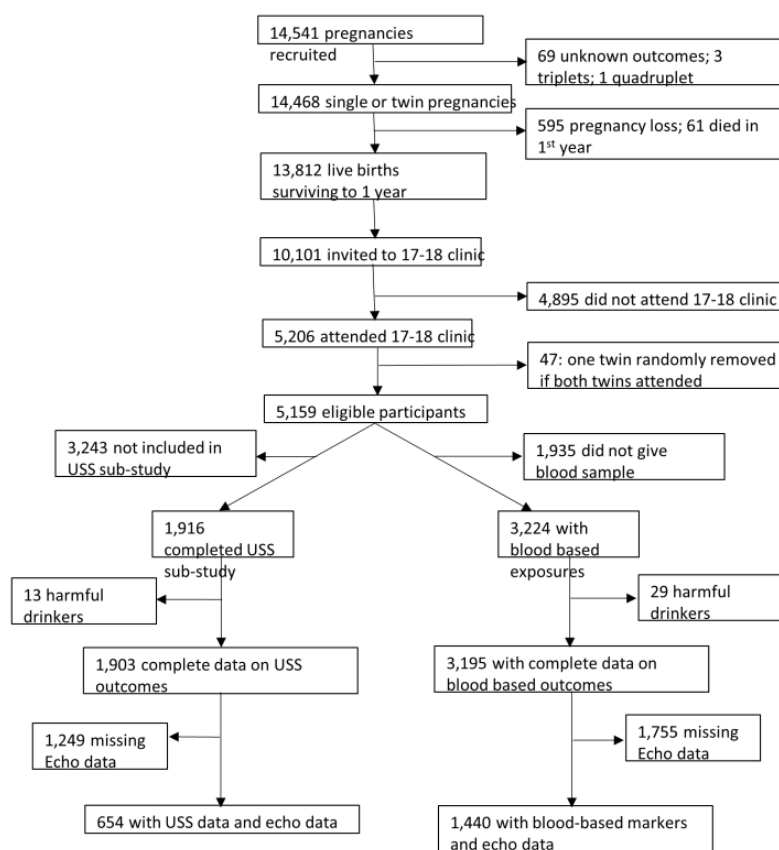


Figure 1 Participants' flow diagram through the study and the numbers included in each of the main analyses.

indicate they had hepatic disease, or were known to influence liver function.

Ethical approval for this study was obtained from the ALSPAC Law and Ethics Committee and the Local National Health Service Research Ethics Committee. All participants provided written informed consent.

Blood-based outcomes and liver ultrasound scans

For both the blood-based analyses and liver USS participants were fasted overnight, for those attending clinic in the morning, or for a minimum of 6 hours, for those attending clinic after lunch.

Assessment of blood-based measures

Fasting blood samples were immediately spun and frozen at -80°C . Measurements were assayed shortly (3-9 months) after samples were taken with no previous freeze-thaw cycles. All assays were completed in the same laboratory at the University of Glasgow. ALT, AST and GGT were measured by an automated analyser with enzymatic methods.

Liver ultrasound scans

A detailed description of the liver ultrasound scans has been published previously [27]. Briefly, upper abdominal USS was completed by one of four trained sonographers using a Siemens Acuson S2000 USS system, with the participant at rest in the dorsal decubitus position. Echogenicity (our marker of liver fat) was assessed during deep inspiration and recorded as present, absent or uncertain according to established protocols using the right kidney as the reference organ [28]. All participants were classified as having absent or present echogenicity; no 'uncertain' echogenicity was noted.

Acoustic radiation force impulse-imaging (ARFI) of the right lobe of the liver was used to measure liver stiffness (or fibrosis), using standard protocols [29,30]. ARFI (measured as shear velocity in meters/second [m/s]) was assessed six times with a gap of at least 1 minute between each measurement. The highest and lowest of these measurements were excluded and the Siemens Acuson S2000 system produced a mean of the remaining four measurements. In the analyses we have used the mean of four measurements after the highest and lowest velocities (of the six taken) were removed. When both right and left lobe values were available the lowest mean of the two has been used.

Assessment of cardiac structure and function

Echocardiography was performed using a HDI 5000 ultrasound machine (Phillips) equipped with a P4-2 Phased Array ultrasound transducer by one of two echo cardiographers using a standard examination protocol. All measurements were made according to American Society of Echocardiography (ASE) guidelines [31] and left ventricular mass and relative wall thickness (RWT) were calculated using validated equations. To account for differences in body size, left ventricular mass and left atrial size (anteroposterior dimension) were indexed to height^{2.7} (LVMI^{2.7} and LAI^{2.7}) [32]. Pulsed Doppler examination of transmitral flow was recorded from the apical four chamber view. For left ventricular measurements the sample volume was positioned between the mitral annulus and the tips of the

mitral leaflets with the position adjusted to maintain the sample volume at an angle as near parallel to transmitral flow as possible with the participant in passive end expiration. The peak flow velocities of the early (E) and atrial (A) transmitral inflow pattern were measured from three cardiac cycles displaying the highest measurable velocity profiles. The ratio of the early and atrial peak flow velocities (E/A) were calculated as a measure of left ventricular diastolic function. Similar measurements were also made at the tricuspid valve. Tissue Doppler echocardiography (TDE) was performed in the 4 chamber view on the lateral left ventricular wall to obtain peak myocardial wall velocities in systole (s') and early and late diastole (e' and a' respectively). Data were acquired with the beam parallel to the wall of interest and with settings optimized to ensure no over-gain of the low velocity signals. A 5 mm sample volume was placed at the level of the mitral valve annulus and still images of 8-10 cardiac cycles were recorded. On-going quality control was performed throughout the study and reproducibility of echocardiographic measurement was assessed by recalling 30 participants and repeating their measurements. The intra-class correlation of repeated echocardiographic measurements was excellent: 0.75 to 0.93 (intra-observer) and 0.78 to 0.93 (inter-observer).

Assessment of other variables

Parental occupation was used to derive household occupational social class, with each household assigned the highest parental occupational (classes I (professional / managerial) to V (unskilled manual workers), using the 1991 British Office of Population and Census Statistics (OPCS) classification). The participant's age was calculated in months from their date of birth and date of attendance at the clinic assessment. Height was measured without shoes to the nearest 0.1cm using a Harpenden stadiometer. A Lunar Prodigy narrow fan beam densitometer was used to perform a whole body dual-energy X-ray absorptiometry (DXA) scan from which lean and fat mass were measured. Puberty information was collected at age 17 by postal questionnaire based on the Tanner staging system. Pubertal stage was based on pubic hair staging for male participants and pubic hair and breast staging for females. If both were available then the higher grade was used. Data on daily cigarette smoking was collected via postal questionnaire at age 16.5 years.

Statistical analysis

All analyses were conducted using Stata version 13.0 MP2 (Stata Inc., TX, USA). Outcome variables of cardiac structure and function that were right skewed were log transformed so that residuals in models would be approximately normal. For these outcomes we back-transformed the coefficients and present them as a % mean difference per unit exposure. A series of multivariable regression models were constructed in order to examine the associations between continuous measures of liver pathology (ALT, GGT, AST and USS assessed liver stiffness) and cardiac structure and function and to explore the impact of adjustment for potential confounding factors. In the basic model we controlled for age and sex (model 1). In the confounder adjusted model we additionally adjusted for household social class, pubertal stage, smoking and alcohol intake and DXA-assessed fat

mass (and height and height-squared to remove any association of fat mass with height) (model 2). Results are presented as mean differences; for naturally logged outcome variables, coefficients were back transformed and multiplied by 100 to give a difference in means presented as a percentage. In sensitivity analyses, we examined whether adjustment for BMI instead of DXA-assessed fat mass altered results (model 2). Age and sex adjusted outcome measures were compared for participants with USS diagnosed fatty liver fat (N = 13) and without.

Dealing with missing data and additional analyses

Of the eligible participants (those included in the liver ultrasound study and/or blood-based markers available, not classed as consistent harmful drinkers, with at least one measure from the echocardiography), a proportion had missing data on any of exposures, outcomes and potential confounding factors (extent of missing for any single variable included in analyses varied from 0-37.4% (Table S1,S2). To increase efficiency and minimise selection bias we used multivariate multiple imputation to impute missing data for any of the eligible participants with missing data. We included all exposures, co variables, outcomes and potential predictors of missing data in the imputation. We generated 40 imputed datasets that were combined by Rubin's rules [33].

RESULTS

Table 1 shows the characteristics of participants who were included in either the blood-based outcome analyses and/or USS based outcomes. The prevalence of USS fatty liver was 2.2% (N = 13). The distribution of characteristics in the observed dataset was similar to those in the imputed datasets (Supplementary web Table S1,S2).

Table 2 shows the multivariable associations of blood-based markers of NAFLD and shear velocity (a measure of liver stiffness) with measures of cardiac structure and function. Higher ALT, AST, GGT and shear velocity were associated with higher LVMI in model 1, but, with the exception of GGT, these associations attenuated to the null in the confounder adjusted model (model 2). This attenuation, which also affected the association of GGT, was largely due to adjustment for fat mass.

There was weak evidence of a positive association of shear velocity with relative wall thickness (RWT) in both models but no strong evidence of associations of ALT, AST or GGT with RWT (Table 2). GGT and shear velocity were inversely associated with s' in both models. There was no evidence of associations of ALT and AST with s', or for any of the exposures with mid wall fractional shortening and ejection fraction.

ALT, AST, GGT and shear velocity were positively associated with LAI (indexed to height^{2.7}) in model 1, but again attenuated in the confounder adjusted model, largely due to adjustment for fat mass.

Weak evidence of positive associations between GGT and E/e' and of shear velocity with mitral E/A emerged only in model 2, i.e. with adjustment for confounders. ALT, AST and GGT were positively associated with left ventricular diastolic diameter in model 1, but attenuated to the null in the confounder adjusted

model, largely due to the confounding effect of fat mass. No associations between liver markers and e' were observed.

There were no notable differences in the associations of markers of NAFLD with measures of cardiac structure and function when BMI (instead of fat mass) was used as a measure of adiposity (data not shown but available on request). We also examined (post hoc) whether height was a confounder in associations between measures of liver health and LVMI and LAI, but when height (but not fat mass or BMI) was accounted for, associations remained essentially unaltered.

There was no evidence of a difference in any measures of cardiac structure and function between adolescents with (N = 13) and without USS fatty liver (N = 641) (Table 3).

DISCUSSION

We conducted a detailed analysis of the associations of several markers of NAFLD with a range of measures of cardiac structure and function assessed at the same time. Of 50 tested associations, there was statistical evidence to support only three (6%) after adjustment for confounders (i.e. very similar to what we would expect by chance with a 5% significance level). Fat mass (or alternatively BMI) was the most important confounder, resulting in marked attenuation of any age and sex adjusted estimate. Taken together these findings suggest that NAFLD is not an independent (of adiposity) determinant of cardiac structure or function in adolescence.

Of the three associations that were observed in confounder adjusted models, it is possible that associations of GGT (but not of ALT and AST) with LVMI and s' reflect extra hepatic sources of GGT consistent with its possible role as a biomarker of cardiovascular disease [34]. Associations between shear velocity and s' may be due to shear velocity being a measure of liver stiffness and thus reflecting more severe NAFLD. However, GGT and shear velocity were not consistently associated with other measures of cardiac structure and function and given that these three associations are 6% of the 50 tested, and we would expect 5% by chance alone, we should assume that these are chance findings unless replicated in other studies.

Obesity is closely related to adverse cardiac structure and function changes even in adolescents and young adults [35]. Studies in children and adolescents have reported associations between obesity and cardiac remodelling, including increased left atrial volume, left ventricular mass indexed to height and left ventricular diastolic volume [36]. In our population of adolescents, measures of NAFLD were largely not associated with cardiac structure and function independently of total fat mass or BMI.

Some cross-sectional studies in adult populations, but not all [18], have reported positive associations of NAFLD with cardiac measures, including left ventricular diastolic function [7,8] and left ventricular hypertrophy [9] even after adjusting for adiposity. It is possible that the discrepancy between results of studies in adults and our own is due to youth of the participants and/or the relatively short exposure time; perhaps exposure NAFLD has a cumulative effect over time and therefore its adverse effects on cardiac structure and function emerge later in life.

Table 1: Characteristics of participants with available data on USS-based and blood-based measures of NAFLD.

Characteristic	USS-based measures of NAFLD		Blood-based measures of NAFLD	
	N	Mean (SD) or median (IQR) or N (%) as indicated	N	Mean (SD) or median (IQR) or N (%) as indicated
Male (% , N)	654	268 (41.0)	1,440	710 (49.3)
Age, months (mean, SD)	654	213.7 (4.5)	1,440	212.3 (4.0)
Manual social class, (% , N)	566	87 (15.4)	1,279	161 (12.6)
Post puberty, (% , N)	409	353 (86.3)	955	810 (84.8)
Smoker, (% , N)	463	41 (8.9)	1,060	96 (9.1)
Alcohol intake (AUDIT score), (% , N)				
0-7	617		1,380	
8-15		404 (65.5)		867 (62.8)
16+		189 (30.6) 24 (3.9)		456 (33.0) 57 (4.1)
BMI, kg/m ² (median, IQR)	654	22.4 (20.3, 25.2)	1,440	22.0 (20.1, 24.7)
Fat mass, kg (median, IQR)	641	17.4 (119, 24.6)	1,407	16.1 (10.2, 23.2)
Height, cm (mean, SD)	636	170.4 (9.6)	1,414	171.9 (9.4)
ALT, U/l (median, IQR)	470	15.3 (12.0, 19.3)	1,440	14.7 (11.7, 19.2)
AST, U/l (median, IQR)	470	19.3 (16.8, 22.8)	1,440	19.4 (16.6, 23.1)
GGT, U/l (median, IQR)	469	16.0 (13.0, 20.0)	1,439	16.0 (13.0, 21.0)
Ultrasound fatty liver, (% , N)	593	13 (2.2)		n/a
Ultrasound shear velocity, m/sec (median, IQR)	598	1.2 (1.1, 1.3)		n/a
Left ventricular mass indexed to height ^{2.7} , g/m ^{2.7} (mean, SD)	633	28.3 (5.9)	1,403	29.1 (6.2)
Relative wall thickness (mean, SD)	650	0.4 (0.05)	1,429	0.4 (0.1)
Average lateral wave, s', cm/s (median, IQR)	634	8.5 (7.4, 9.8)	1,405	8.4 (7.3, 9.8)
Midwall fractional shortening, % (mean, SD)	650	15.9 (2.2)	1,429	15.5 (2.2)
Ejection fraction, %, (mean, SD)	651	66.5 (6.4)	1,430	66.3 (6.5)
Lateral E/e' ratio (median, IQR)	630	4.8 (4.2, 5.3)	1,389	4.8 (4.2, 5.4)
Mitral E/A (median, IQR)	634	1.9 (1.6, 2.1)	1,399	1.9 (1.6, 2.1)
Left atrial size indexed to height, cm/m (median, IQR)	584	0.8 (0.7, 0.9)	1,273	0.7 (0.7, 0.8)
e', cm/s (median, IQR)	634	13.3 (11.7, 15.0)	1,405	13.1 (11.5, 14.9)
Left ventricular diastolic diameter, cm (mean, SD)	651	4.5 (4.2, 4.8)	1,430	4.5 (0.4)

^a: P-value for the null hypothesis of no difference compared to those included in either analysis of blood based or USS outcomes group (reference group)
Abbreviations: USS: Ultrasound Scan; ALT: Alanine Amino Transferase; AST: Aspartate Amino Transferase; GGT: Gamma-Glutamyl Transferase; IQR: Interquartile Range; SD: Standard Deviation; N/A: Not Applicable

Table 2: Multivariable associations of shear velocity (N=654) and blood-based markers (N=1,440) of non-alcoholic fatty liver disease with measures of cardiac structure and function.

	Model 1		Model 2	
	Mean differences or % mean differences (95% CI)	P value	Mean differences or % mean differences (95% CI)	P value
Ventricular structure				
Left ventricular mass indexed to height^{2.7} (g/m^{2.7})				
ALT per 10 U/l	0.57 (0.29, 0.85)	<0.001	0.11 (-0.25, 0.27)	0.94
AST per 10 U/l	0.39 (0.02, 0.76)	0.04	0.12 (-0.22, 0.46)	0.48
GGT per 10 U/l	0.59 (0.37, 0.81)	<0.001	0.29 (0.09, 0.50)	0.005

Table 2: Multivariable associations of shear velocity (N=654) and blood-based markers (N=1,440) of non-alcoholic fatty liver disease with measures of cardiac structure and function.

	Model 1		Model 2	
	Mean differences or % mean differences (95% CI)	P value	Mean differences or % mean differences (95% CI)	P value
Shear velocity per SD (m/sec)	0.67 (0.20, 1.15)	0.006	0.01 (-0.43, 0.44)	0.98
Relative wall thickness				
ALT per 10 U/l	-0.0001 (-0.0028, 0.0026)	0.94	-0.0009 (-0.0037, 0.0019)	0.52
AST per 10 U/l	-0.0016 (-0.0051, 0.0020)	0.39	-0.0019 (-0.0054, 0.0017)	0.31
GGT per 10 U/l	0.0013 (-0.0008, 0.0034)	0.23	-0.0009 (-0.0013, 0.0030)	0.43
Shear velocity per SD (m/sec)	0.0036 (-0.0002, 0.0074)	0.07	0.0032 (-0.0008, 0.0072)	0.12
Systolic function				
s' (%^a)				
ALT per 10 U/l	0.18 (-0.77, 1.14)	0.71	0.41 (-0.56, 1.40)	0.41
AST per 10 U/l	-0.39 (-1.67, 0.90)	0.55	-0.28 (-1.56, 1.02)	0.68
GGT per 10 U/l	-0.91 (-1.64, -0.16)	0.02	-0.83 (-1.58, -0.08)	0.03
Shear velocity per SD (m/sec)	-2.71 (-4.31, -1.08)	0.001	-2.45 (-4.11, -0.76)	0.005
Midwall fractional shortening (%)				
ALT per 10 U/l	-0.10 (-0.29, 0.09)	0.55	0.02 (-0.08, 0.12)	0.71
AST per 10 U/l	0.08 (-0.05, 0.21)	0.22	0.09 (-0.04, 0.23)	0.16
GGT per 10 U/l	-0.05 (-0.13, 0.03)	0.21	-0.02 (-0.10, 0.06)	0.63
Shear velocity per SD (m/sec)	-0.02 (-0.19, 0.16)	0.86	0.04 (-0.14, 0.22)	0.67
Ejection fraction (%)				
ALT per 10 U/l	0.02 (-0.32, 0.27)	0.88	0.05 (-0.26, 0.35)	0.77
AST per 10 U/l	0.08 (-0.30, 0.47)	0.67	0.09 (-0.30, 0.48)	0.65
GGT per 10 U/l	-0.03 (-0.27, 0.20)	0.77	0.01 (-0.22, 0.25)	0.92
Shear velocity per SD (m/sec)	0.18 (-0.34, 0.70)	0.50	0.28 (-0.26, 0.82)	0.31
Diastolic function				
E/e' (%^a)				
ALT per 10 U/l	-0.22 (-1.14, 0.70)	0.64	0.26 (-0.69, 1.22)	0.59
AST per 10 U/l	-0.24 (-1.51, 1.04)	0.71	-0.06 (-1.33, 1.23)	0.93
GGT per 10 U/l	0.40 (-0.33, 1.13)	0.29	0.70 (-0.04, 1.45)	0.07
Shear velocity per SD (m/sec)	0.68 (-0.93, 2.31)	0.41	1.01 (-0.65, 2.70)	0.23
Mitral E/A(%^a)				
ALT per 10 U/l	-0.79 (-1.69, 0.11)	0.09	-0.16 (-1.08, 0.77)	0.74
AST per 10 U/l	0.14 (-1.08, 1.38)	0.82	0.40 (-0.82, 1.64)	0.52
GGT per 10 U/l	-0.64 (-1.34, 0.07)	0.08	-0.29 (-1.00, 0.43)	0.43
Shear velocity per SD (m/sec)	0.75 (-0.85, 2.39)	0.36	1.56 (-0.10, 3.25)	0.07
Left atrial size indexed to height(%^a)				
ALT per 10 U/l	1.37 (0.64, 2.11)	<0.001	0.25 (-0.32, 0.82)	0.40
AST per 10 U/l	1.28 (0.30, 2.28)	0.01	0.52 (-0.22, 1.27)	0.17
GGT per 10 U/l	0.97 (0.39, 1.55)	0.001	0.37 (-0.07, 0.81)	0.10
Shear velocity per SD (m/sec)	2.53 (1.18, 3.89)	<0.001	1.13 (0.13, 2.13)	0.36
e' (%^a)				
ALT per 10 U/l	0.68 (-0.27, 1.63)	0.16	0.43 (-0.54, 1.42)	0.39
AST per 10 U/l	0.30 (-0.98, 1.59)	0.65	0.12 (-1.16, 1.42)	0.85
GGT per 10 U/l	-0.23 (-0.97, 0.52)	0.54	-0.39 (-1.14, 0.37)	0.31
Shear velocity per SD (m/sec)	0.33 (-1.32, 2.00)	0.70	0.08 (-1.60, 1.80)	0.93

Table 2: Multivariable associations of shear velocity (N=654) and blood-based markers (N=1,440) of non-alcoholic fatty liver disease with measures of cardiac structure and function.

	Model 1		Model 2	
	Mean differences or % mean differences (95% CI)	P value	Mean differences or % mean differences (95% CI)	P value
Left ventricular diastolic diameter (cm)				
ALT per 10 U/l	0.02 (0.00, 0.04)	0.02	0.00 (-0.02, 0.02)	0.85
AST per 10 U/l	0.03 (0.00, 0.05)	0.03	0.02 (-0.00, 0.04)	0.07
GGT per 10 U/l	0.02 (0.01, 0.03)	0.01	0.01 (-0.01, 0.02)	0.25
Shear velocity per SD (m/sec)	0.02 (-0.01, 0.05)	0.22	-0.01 (-0.04, 0.02)	0.47
ALT: Alanine Amino Transferase; AST: Aspartate Amino Transferase; GGT: Gamma-Glutamyl Transferase The null value for all analyses is 0. Model 1 (basic model): age at time of assessment and gender Model 2 (confounder adjusted model): as model 1 plus social class and puberty, smoking, alcohol intake, fat mass, height and height squared *For these results the outcome was log-transformed and the regression coefficient back transformed so that the results are the % difference of outcome per unit of exposure.				

Table 3: Age and gender adjusted values of cardiac structure and function measures by USS-determined fatty liver (N=654).

	Mean or geometric means of cardiac structure and function measures (95% CI) by whether the participant had USS-determined fatty liver		P value
	Yes (N=13)	No (N=641)	
Ventricular structure			
Left ventricular mass indexed to height ^{2,7} , g/m ^{2,7}	30.4 (26.9, 34.0)	29.3 (27.9, 28.8)	0.42
Relative wall thickness	0.4 (0.4, 0.4)	0.4 (0.4, 0.4)	0.62
Systolic function			
s', cm/s	8.3 (7.5, 9.1)	8.5 (8.3, 8.6)	0.43
Midwall fractional shortening, %	15.5 (14.4, 16.5)	15.9 (15.7, 16.1)	0.69
Ejection fraction, %	65.9 (62.7, 69.0)	66.5 (66.0, 67.0)	0.96
Diastolic function			
E/e'	5.0 (4.5, 4.8)	4.7 (4.7, 4.8)	0.21
Mitral E/A	1.8 (1.7, 2.0)	1.9 (1.8, 1.9)	0.51
Left atrial size indexed to height, cm/m	0.8 (0.7, 0.8)	0.8 (0.7, 0.8)	0.40
e', cm/s	13.0 (11.7, 14.4)	13.3 (13.1, 13.5)	0.66
Left ventricular end diastolic diameter, cm	4.7 (4.5, 4.9)	4.5 (4.5, 4.6)	0.78
CI: confidence intervals			

To our knowledge this is the first study that has considered the association between NAFLD and cardiac structure and function in a general population sample of adolescents; other studies in adolescents have been mainly in selected obese populations [22,23,37-39]. Similar to our results the majority of these studies reported a crude positive association of NAFLD with LVMI [22,23,37,38] Singh et al., [39] compared 3 groups of adolescents; lean adolescents, obese adolescents without NAFLD and obese adolescents with NAFLD (determined by magnetic resonance spectroscopy). They found greater LVMI in obese (n = 30) compared to lean (n = 14) adolescents at a median age of 15 years, but no strong evidence of a difference between obese participants with (n = 15) and without NAFLD (n = 15), possibly indicating that the association was driven by adiposity and not by the presence of NAFLD.

Other studies in selected obese samples have reported associations between NAFLD and cardiac structure even after

adjusting for adiposity and other CVD risk factors. Pacifico et al., [23] reported a greater E/e' ratio in obese NAFLD participants (N = 54) compared to obese non-NAFLD (N = 54) and lean participants (N = 18) in unadjusted analysis. In multivariable analysis, E/e' was dichotomised (using the median value of 6.83) and after adjusting for age, sex, puberty, BMI, abdominal fat, systolic and diastolic blood pressure, triglycerides, HDLc, and whole-body insulin sensitivity index, NAFLD remained associated with high E/e'. In a study examining the association of NAFLD in obese adolescents mean age 13.5 years, (n = 97) with LVM, age, total cholesterol, AST and insulin sensitivity were associated with LVM in multivariable analysis [22]. The results from these two studies differ from our findings, which may reflect the differences in the study samples' adiposity levels.

The work presented here extends earlier work conducted using data from the same sample of adolescent participants in the ALSPAC, which demonstrated that association of markers of

NAFLD, including USS-determined fatty liver with central and peripheral systolic blood pressure, diastolic blood pressure, and mean arterial pressure were confounded by adiposity [40], whilst associations of USS fatty liver with greater insulin resistance and dyslipidemia, persisted even after adjusting for measures of adiposity [27].

STRENGTHS AND LIMITATIONS

This study is the largest to date that has examined the association of several measures of liver health with cardiac structure and function in an unselected cohort of healthy adolescents. Whilst the proportion of participants with USS determined NAFLD in our cohort is lower than that observed in another study of healthy 11-13 year olds in Southern Italy [41] it is within the range of reported estimates [42,43]. The number of participants with NAFLD in our study is small ($n = 13$) and therefore the lack of associations seen for that exposure after adjustment may be due to lack of statistical power.

Although USS-determined NAFLD is not the gold-standard, undertaking biopsies to determine NAFLD status in a large healthy population, as described in this study, would be unethical. In general, USS, has a sensitivity of 85-90% and specificity of 70-85% for detecting liver fat of at least 10%, but lower sensitivity and specificity for lower levels of fat [44]. Therefore, it is likely that NAFLD in our cohort reflect the prevalence of the more moderate to severe end of the spectrum. Moreover, studies in children have shown USS to accurately identify moderate to severe steatosis compared with liver biopsy [45]. The ARFI measure of liver stiffness used in our study is a relatively new measure, but has been validated in a small number of clinical studies [46,47]. Cardiac structure and function were assessed by ultrasound which does not detect sub-clinical changes in cardiac function, such as cardiac torsion. The majority of this population are of European origin and we cannot assume that results generalise to other populations. Finally, our study is cross-sectional and further follow-up of this cohort will be valuable in determining whether associations change over time.

CONCLUSION

Our results suggests that in a general population of adolescents, markers of NAFLD are not associated with cardiac structure or function once the confounding effect of adiposity has been taken into account. These results support the importance of weight management in the preservation of cardiac function, but do not support specialist approaches targeting liver lipid content for optimising cardiac function in this population.

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AUTHOR CONTRIBUTIONS

AF obtained funding and designed the study. SP conducted analyses and wrote the first draft of the manuscript. ADH, DAL, NC, DLSF, MC, CD, NS and AF revised the manuscript critically for important intellectual content. All authors approved the final version. SP acts as the guarantor for this manuscript.

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